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THE STRUCTURE AND SYNTHESIS OF SCLEROLIDE[1]

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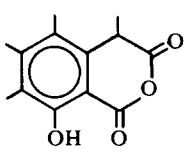
Abstract—The structure elucidation and synthesis of sclerolide, a co-metabolite of sclerin is described.

FROM the submerged culture of *Sclerotinia libertiana,* a minute amount of a crystalline lactonic metabolite, m.p. 163°, was isolated in addition to sclerin (**1**).[2] This compound, designated as sclerolide,[1] has been found to show a plant growth promoting effect analogous to sclerin.[3] Recently the isolation of further three co-metabolites, sclerotinin A (**3**),[4] sclerotinin B (**4**)[4, 5] and sclerone (**5**)[6] from the other strain of the fungus, *Sclerotinia sclerotiorum* has been reported. The structure determination and synthesis of sclerolide (**2**) are the subject of this paper.



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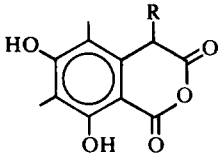

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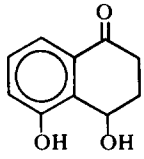

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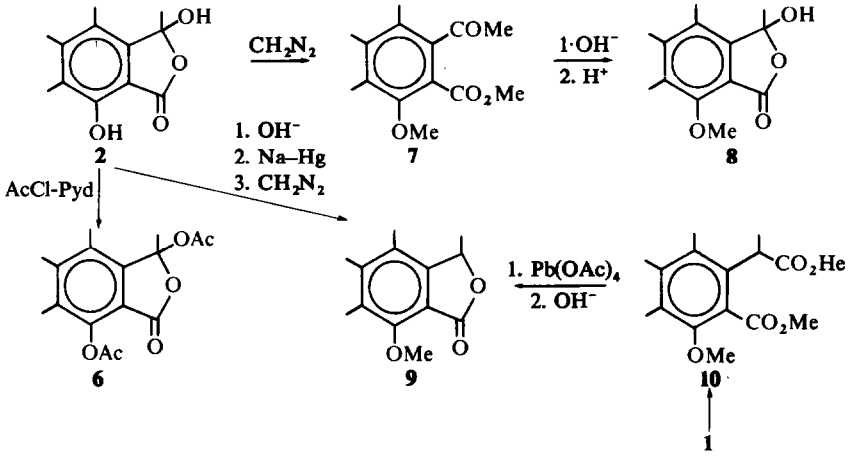
Sclerolide (**2**) has a molecular formula of $C_{12}H_{14}O_4$, one carbon being lost from sclerin (**1**). It gives a blue colour in the ferric chloride test and, upon acetylation, affords a diacetate **6**. In the IR spectrum, sclerolide (**2**) exhibits an OH band at 3420 cm^{-1} and a CO stretching band at 1720 cm^{-1}. The latter band shifts to 1780 cm^{-1} in the spectrum of the diacetate **6**. These data indicate the presence of two OH groups, at least one to be phenolic, and a chelated γ-lactone system. A close resemblance between sclerolide (**2**) and sclerin (**1**) was observed in their UV and NMR spectra.[7] Thus sclerolide (**2**) showed three UV absorption maxima at 213 (ε 16300), 250 (7700) and 313 mμ (4300) comparable with those of sclerin (**1**) at 215·5 (ε 23100), 263 (7900) and 333 mμ (3500).* In the NMR spectrum sclerolide (**2**) exhibits a singlet at 2·31 τ due to an OH proton and four Me singlets at 7·69, 7·76, 7·82 and 8·11 τ. The only significant change from the spectrum of sclerin (**1**) is that the A_3X pattern signals due to $CH_3\underset{|}{C}H$— in **1** are replaced by a Me singlet at 8·11 τ in **2**. The presence of this signal in rather low field indicates that this Me group is linked to a C atom bearing a negative substituent. Incidentally, the NMR spectrum of the diacetate **6** shows the acetate signals at 7·60 and 8·01 τ, which are assigned to phenolic and

* Blue shift of the principal electron transfer band due to the ring strain effect in the five-membered ring is observed in the former, cf. the footnote of Ref. 7, p. 2346.

alcoholic OH protons respectively. Based on these facts scleroide has the structure **2**. This is in accordance with the view that **2** could be biogenetically derived by oxidative decarboxylation of sclerin (**1**)

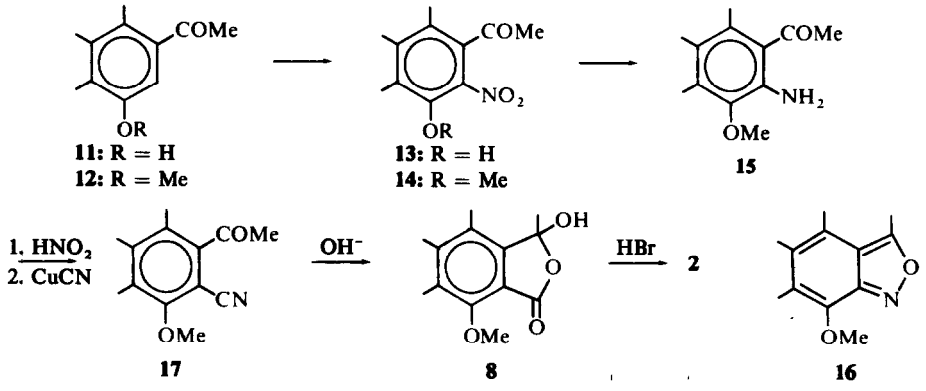
Methylation of sclerolide affords a normal methyl ester **7**, which shows CO stretching bands at 1720 and 1695 cm^{-1} in the IR spectrum. The presence of an acetyl group in **7** is substantiated by the NMR spectrum, which exhibits a 3-proton singlet at 7·52 τ in addition to the aromatic Me signals at 7·79, 7·77 and 7·84 τ. Alkaline hydrolysis of **7** resulted in the reformation of lactol ring to yield the compound **8**. In agreement with the assigned structure, the lactol **8** has IR peaks at 3370 and 1750 cm^{-1} and UV maxima at 213 (ε 34600), 250 (9500) and 300 mμ (2700). The signal due to the



tertiary Me group is at 8·16 τ in the NMR spectrum. Eventually sclerolide (**2**) was chemically correlated with sclerin (**1**) in the following manner. When an alkaline solution of sclerolide was reduced with sodium amalgam and the product methylated, a 5-membered lactone **9** was obtained. The product **9** had already been derived from sclerin (**1**) via 2-(5-carbomethoxy-4-methoxy-1,2,3-trimethylphenyl)propionic acid (**10**)[7] and, although it has higher m.p. (13°) than the compound available from **10**, the identity of both compounds was confirmed by comparison of their IR spectra. The reason for this deviation in the m.ps could be due to the optical activity. The product obtained above must be racemic, while the lactone **9** derived from sclerin could be partially optical active.* Finally the proposed structure **2** for sclerolide was verified by synthesis, starting from 5-hydroxy-2,3,4-trimethylacetophenone (**11**).[9] After an attempt to introduce a carboxyl function directly on the methyl ether **12** by Friedel–Crafts procedure (oxalyl chloride–$AlCl_3$),[10] our attention was turned to the use of the Sandmeyer reaction. Nitration of **11** in a mixture of carbon tetrachloride and acetic acid afforded 5-hydroxy-6-nitro-2,3,4-trimethylacetophenone (**13**) in a good yield. The nitration of the corresponding methyl ether **12** in the same way resulted in the

* The acid **10** used for the preparation of the lactone **8** was crude oily material obtained from the mother liquor of crystallization.[7] Therefore, it is presumed that the acid **10** in this case could be optically active. cf. the footnote of Ref. 7, p. 2345. Optical retention in the oxidative decarboxylation with lead tetraacetate has been reported.[8]

partial demethylation and produced a mixture of **13** and **14**. Although demethylation during nitration was observed previously,[11] the effect of steric compression is no doubt responsible for the extreme ease of the ether cleavage in such a crowded molecule as **14**. The methylation of **13** was achieved by treating its sodium salt with dimethyl sulphate.[12] The reduction of 6-nitro-5-methoxy-2,3,4-trimethylacetophenone (**14**) to



the corresponding amine **15** was a problem because of formation of the anthranil **16**. When **14** was treated with stannous chloride, the yield of the desired aminoacetophenone **15** varied from 61% to a trace. The rest of the product was the anthranil **16**. Catalytic reduction over palladium or reaction with sodium hydrosulphite gave similar results. However this situation was relieved by the fact that the anthranil **16** was converted to the aminoacetophenone **15** by the treatment with metallic tin and conc hydrochloric acid at 100°. The aminoacetophenone **15** was transformed to the cyanoacetophenone **17** by means of the Sandmeyer reaction. 5-Methoxy-2,3,4-trimethylacetophenone (**12**) was obtained as by-product. Alkaline hydrolysis of the cyanoacetophenone **17** yielded the lactol **8**, which was identical with the compound derived from sclerolide (**2**). Demethylation of the lactol **8** by treatment with hydrobromic acid gave sclerolide (**2**). The identity of the natural and synthetic products was confirmed by mixed m.p. and comparison of their IR spectra.

EXPERIMENTAL

M.ps were uncorrected. IR spectra were recorded on Nujol mull on a Nippon Bunko IR-S spectrophotometer and UV spectra were measured in EtOH soln on a Hitachi EPS-2 recording spectrophotometer. The NMR spectra were run on a JNMC-60 spectrometer in $CDCl_3$. Chemical shifts are expressed in τ-values using TMS as internal standard.

Sclerolide (**2**)

The sample provided by Prof. Y. Satomura was recrystallized from a mixture of $CHCl_3$ and ether to give prisms, m.p. 162–163°; ν_{max} 3420, 1720 cm^{-1}; λ_{max} 213 (ε 16,300), 250 (7700), 313 mμ (4300); NMR: 2·31 (s., —OH), 7·69, 7·76, 7·82 (each s., 3ArMe), 8·11 (s., —$\overset{|}{C}$Me). It gave blue colour in $FeCl_3$ test (MeOH). (Found: C, 64·97; H, 6·41. $C_{12}H_{14}O_4$ requires: C, 64·85; H, 6·35%).

Sclerolide diacetate (**6**)

To an ice-cooled soln of sclerolide (100 mg) in anhyd pyridine (1·2 ml) acetyl chloride (0·5 ml) was added under swirling. After the mixture was kept at 0° for 1 hr and at room temp for 3 hr, usual working up afforded **6**. It crystallized from ether as rectangular prisms, m.p. 147–147·5°; ν_{max} 1780, 1760 cm^{-1}; λ_{max} 211·5 (ε 47,000), 250·5 (11,000), 296 (2600), λ_{infl} 290 mμ (2500); NMR: 7·60 (s., AcOAr), 7·72, 7·74, 7·85 (each s., 3ArMe), 8·01 (s., AcOR), 8·09 (s., —CMe). (Found: C, 62·93; H, 5·92. $C_{16}H_{18}O_6$ requires: C, 62·74; H, 5·92%).

Conversion of sclerolide to the lactol (**8**)

Sclerolide (50 mg) in MeOH was methylated overnight with excess ethereal CH_2N_2. The product was recrystallized twice from light petroleum to give *methyl 2-acetyl-6-methoxy-3,4,5-trimethyl benzoate* (**7**) as prisms, m.p. 56–57°; ν_{max} 1720, 1695 cm^{-1}; λ_{max} 213 (ε 19,800), 291 (1700), λ_{infl} 245 mμ (6300); NMR: 6·16 (s., —OMe), 6·26 (s., —CO_2Me), 7·52 (s., ArCOMe), 7·79, 7·77, 7·84 (each S., 3ArMe). (Found: C, 67·45; H, 7·58. $C_{14}H_{18}O_4$ requires: C, 67·18; H, 7·25%). The methyl ester **7** (20 mg) in MeOH (2 ml) was treated with 2N NaOH (1 ml) under refluxing for 2 hr. The product isolated was recrystallized from a mixture of ether and light petroleum to afford *the lactol* **8** as crystals, m.p. 142–144°; ν_{max} 3370, 1750 cm^{-1}; λ_{max} 213 (ε 34,600), 250 (9500), 300 mμ (2700); NMR: 5·27 (s., —OH), 6·14 (s., —OMe), 7·63, 7·68, 7·84 (each s., 3ArMe), 8·16 (s., —CMe). (Found: C, 66·07; H, 6·93. $C_{13}H_{16}O_4$ requires: C, 66·08; H, 6·83%).

Conversion of sclerolide to the 5-membered lactone **9**

After sclerolide (44 mg) in EtOH (1·0 ml) was treated with 1N KOH (0·5 ml) under refluxing for 1 hr; to the cooled soln Na–Hg (3·3% 1·5 g) was added and the mixture kept at room temp with occasional shaking for 2 hr. The reaction mixture was freed from Hg by decantation and acidified with dil HCl. Ether extraction yielded an yellow oil, a part (36 mg) of which was methylated with CH_2N_2. the methylated product was chromatographed on a column of silica gel (0·5 g) and benzene elution gave oily fractions (11 mg) which were crystallized from a mixture of ether and light petroleum to afford *the 5-membered lactone* **9**, m.p. 102–103°. The IR spectrum of this product was superimposable with that of the specimen derived from sclerin.[7]

Reaction of 5-methoxy-2,3,4-trimethylacetophenone (**12**) *with oxalyl chloride*

A soln of **12** (192 mg) and oxalyl chloride (0·48 ml) in tetrachloroethane (1·5 ml) was cooled with ice bath. Under magnetic stirring, anhyd $AlCl_3$ (434 mg) was added in one portion and the mixture was stirred at 0° for 18 hr. The starting material, m.p. 65–66° (94 mg) was recovered unaltered after usual working up and recrystallization from light petroleum.

6-Nitro-5-hydroxy-2,3,4-trimethylacetophenone (**13**)

A mixture of conc HNO_3 (S.G. 1·38, 2·4 ml, 37 mmole) and conc H_2SO_4 (3·6 ml) was added dropwise to a soln of **13** (3·0 g, 17 mmole) in a mixture of CCl_4 (30 ml) and AcOH (60 ml), during 7 min, the inner temp being kept between −5° and 0°. The reaction was continued for further 10 min and stopped by the addition of ice–water. The ether extract was washed with $NaHCO_3$aq and the neutral product (3·99 g) was recrystallized from EtOH to afford **13** as yellow needles, m.p. 99–100° (2·82 g, 80% yield); ν_{max} 3200–2600 (br), 1705, 1530, 1335 cm^{-1}. (Found: C, 58·97; H, 5·98; N, 6·43. $C_{11}H_{13}O_4N$ requires: C, 59·18; H, 5·87; N, 6·28%).

Nitration of 5-methoxy-2,3,4-trimethylacetophenone (**12**)

A soln of **12** (960 mg, 5 mmole) in CCl_4 (10 ml) was cooled down to −12°. To this soln a mixture of conc HNO_3 (S.G. 1·42, 0·9 ml, 15 mmole) and conc H_2SO_4 (1·35 ml) was added during 3 min under vigorous stirring. Meanwhile the inner temp was raised up to −5° and the stirring was continued for further 15 min. The reaction mixture was poured onto ice–water and the product was extracted with ether. The neutral product (1·241 g) was chromatographed on a column of silica gel (15 g) and the crystalline fractions eluted with benzene (394 mg in total) were recrystallized from EtOH to give *5-hydroxy-6-nitro-2,3,4-trimethylacetophenone* (**13**), m.p. 96–98°, identical with the compound obtained above.

5-Methoxy-6-nitro-2,3,4-trimethylacetophenone (**14**)

The hydroxynitroacetophenone **13** (2·821 g, 12·6 mmole) was dissolved in 4N NaOH (4·11 ml, 16·4 mmole) and the soln was evaporated to dryness. The Na salt thus obtained, after complete drying by means of azeotrope distillation with dry benzene, was suspended in dry benzene (28 ml) and treated with Me_2SO_4 (2·82 ml, 22·4 mmol) under refluxing for 3 hr. Usual working up gave 1·035 g of the acidic product, which represented the unchanged starting material, and 2·290 g of the neutral product. The latter as benzene soln was filtered through a column of silica gel to afford, after recrystalliaztion from EtOH, **14** as crystals, m.p. 69–70·5° (1·495 g) and a second crop, m.p. 66–68°; (0·36 g) 62% in total; ν_{max} 1700, 1527, 1357 cm^{-1}. (Found: C, 60·75; H, 6·39; N, 5·84. $C_{12}H_{15}O_4N$ requires: C, 60·75; H, 6·37; N, 5·90%).

Reduction of 5-methoxy-6-nitro-2,3,4-trimethylacetophenone (**14**)

(i) *With $SnCl_2$.* The methoxynitroacetophenone **14** (273 mg, 1·16 mmole) was added in one portion to a stirred soln of $SnCl_2 \cdot 2H_2O$ (1·1 g, 5 mmole) in conc HCl (1·5 ml). The reaction temp was held at 90° for 10 min with external heating. The reaction mixture was treated with 40% NaOH aq and the product was extracted with ether. The organic layer was washed with water several times and, after drying with $MgSO_4$, the solvent was evaporated to leave an oily product (225 mg), which was recrystallized from a mixture of light petroleum and ether to afford the crude **15** as crystals m.p. 74–78° (58 mg). The mother liquor was dissolved in ether and extracted with dil HCl. The HCl extract was made alkaline with conc ammonia and extracted with ether. Evaporation of the solvent left an additional amount (76 mg) of **15**, (145 mg in total, 61% yield) and recrystallized several times from a mixture of ether and light petroleum to afford pure *6-amino-5-methoxy-2,3,4-trimethylacetophenone* (**15**), as colourless crystals, m.p. 90–92°; ν_{max} 3440, 3350, 3240, 1695, 1635 cm^{-1}. (Found: C, 69·59; H, 8·35; N, 6·67. $C_{12}H_{17}O_2N$ requires: C, 69·54; H, 8·27, N, 6·76%). The neutral product (76 mg) obtained from the ether layer was recrystallized from a mixture of light petroleum ether and ether to give *7-methoxy-3,4,5-tetramethylanthranil* **16** as crystals, m.p. 80°; ν_{max} 1635, 1570, 1535 cm^{-1}; λ_{max} 330 mμ (ε 27,600). (Found: C, 70·30; H, 7·29; N, 6·86. $C_{12}H_{15}O_2N$ requires: C, 70·22; H, 7·37; N, 6·82%).

(ii) *With sodium hydrosulphite.* A soln of **14** (112 mg) in EtOH (5 ml) and water (5 ml) was warmed to 60° and added to a soln of $Na_2S_2O_4$ in water (4.2 ml). The mixture was kept at 60–70° for 30 min and then worked up to produce the anthranil **16** (95 mg) with a trace amount of **15**. Treatment of **14** with sodium dithionate in similar way gave an analogous result.

(iii) *Catalytic hydrogenation.* A soln of **14** in EtOH (10 ml) was hydrogenated with prereduced 10% Pd-C in EtOH (10 ml). The absorption of H_2 ceased after 2½ hr (uptake 80 ml at 25°). Usual working up yielded 78 mg of **15** and 114 mg of **16**.

6-Cyano-5-methoxy-2,3,4-trimethylacetophenone (**17**)

The amine **15** (312 mg, 1·65 mmole) in 9N H_2SO_4 (0·56 ml) was diazotized with $NaNO_2$ (137 mg, 1·98 mmole) in water (0·7 ml) at 0° for 20 min. KCN (910 mg, 14 mmole) was added to a warmed soln (60°) of $CuSO_4 \cdot 5H_2O$ (825 mg, 3·3 mmole) in water (3·3 ml) and this soln maintained at 70–80°, was added dropwise to the diazonium salt soln prepared above. After the mixture was heated for further 30 min at 100°, the product was isolated by ether extraction. The neutral product (115 mg) was chromatographed on a column of silica gel (5 g). The earlier benzene eluate (18 mg) was recrystallized from light petroleum to give crystals, m.p. 64–65°, which was identified as **12**. The following fractions (35 mg) eluted by benzene and $CHCl_3$ represented the desired compound **17**, as indicated by the presence of the IR peaks at 2230, 1695 cm^{-1}.

Hydrolysis of 6-cyano-5-methoxy-2,3,4-trimethylacetophenone (**17**)

The cyanoacetophenone **17** (35 mg) was heated under reflux with 15% KOH aq (5 ml) and ethylene glycol (2·5 ml) for 5 hr. The acidic product (26 mg) was recrystallized from a mixture of light petroleum and ether to give **8** as crystals, m.p. 145–146·5°. Further purification of this product by vaccum sublimation (bath temp 130–140°/2 mm) and recrystallization did not change its m.p. Mixing with the compound (m.p. 145–146°) derived from sclerolide did not depress its m.p. and their IR spectra were indistinguishable.

Conversion of the lactol **8** *to sclerolide* (**2**)

The lactol **8** (20 mg) was heated under reflux with HBr (48%, 2 ml) and AcOH (2 ml) for 2 hr. Dilution

with water followed by ether extraction yielded a crystalline product, which was purified by recrystallization and vacuum sublimation. The product, m.p. 158–159° was identical with natural sclerolide (mixed m.p. and IR).

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